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Homochirality and Life

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ABSTRACT: Before the emergence of life, left-handed amino acids (L-enantiomers) were selected and right-handed amino acids (D-enantiomers) were eliminated on the primal earth. Nevertheless, with the progress of analytical methods, D-amino acids have recently been found in higher order living organisms in the form of free amino acids, peptides, and proteins. Free D-amino acids have numerous physiological functions. D-amino acids containing animal peptides are well known as opioid peptides. D-amino acids in protein are related to aging. In this review, we describe the D-amino acids that are present and function as D-amino acid biosystems in our bodies. © 2004 The Japan Chemical Journal Forum and Wiley Periodicals, Inc. Chem Rec 4: 267–278; 2004: Published online in Wiley InterScience (www.interscience.wiley.com) DOI 10.1002/tcr.20020

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Establishment of Homochirality in Life

Amino acids contain one (or more) asymmetric tetrahedral carbon atoms. Therefore, the molecules are two nonsuperposable mirror images, that is, they are right-handed (D-enantiomer) and left-handed (L-enantiomer) structures, as shown in Figure 1. It is considered that equal amounts of D- and L-amino acids existed on primal earth before the emergence of life. However, during the stage of chemical evolution, only L-amino acids were selected for polymerization and formation of peptides and proteins after which life emerged. Although the chemical and physical properties of L-amino acids and D-amino acids are extremely similar except for their optical character, the reasons for the elimination of D-amino acids and why all living organisms are now composed predominantly of L-amino acids are not well known. However, it is clear that only one of the enantiomers could be selected because polymers, which consist of many amino acid diastereomers would not be able to be folded into proper structures in a manner similar to current proteins. Homochirality is essential for the development and maintenance of life. Once the L-amino acid world was established, D-amino acids were excluded from living systems. Consequently, there has been little study of the presence and function of D-amino acids in living organisms.

Early Work

Although homochirality is essential for life, the presence of several D-amino acids in microorganisms was accepted exceptionally about 40 years ago. D-alanine and D-glutamyl residues constitute the cell wall of bacteria and D-valine and D-phenylalanine are present in many antibiotics, such as valinomycin, actinomycin, gramicidin S, and tyrocidines. These amino acids are synthesized by nonribosomal pathways and

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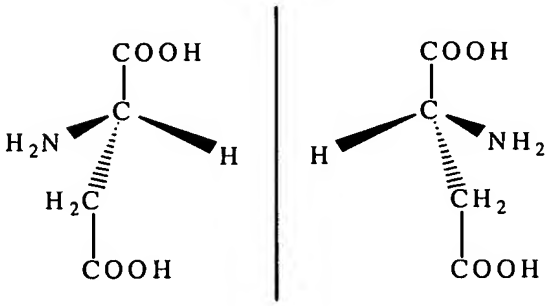

L-Asp D-Asp

Fig. 1. Optical isomers of amino acids.

through multienzyme systems (racemase and amino acid transferase) in microorganisms. The D-amino acids are synthesized and metabolized in biosystems of microorganisms.[1,2] Several species of the Gram-positive bacteria *Bacillus* produce poly gamma-D-glutamate as an extracellular viscous material that is well known as that of fermented soybeans. Although, the existence of D-amino acids was thought to be limited to microorganisms, several free D-amino acids were observed in multicellular organisms such as seaweed, mollusks, and silkworms, which were reviewed in detail by Soda.[3]

Recent Work

In the 1930s, D-amino acid oxidase, which oxidizes D-amino acids, was discovered in mammals. However, since D-amino

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acids were not considered to be present in higher order living systems, the physical meaning of the presence of the enzymes was not been well understood for a long time. In recent years (1980s and 1990s), D-amino acids were detected in a variety of living higher organisms in the form of free, peptide-, and protein-bound D-amino acids. These discoveries depended on the progress of analytical technology for optical isomers of amino acids. In past studies, D-amino acids may not have been detected or were underestimated because of inadequate analytical procedures.

Free D-Amino Acids in Higher Order Organisms

Free D-Serine (Ser) in Mammalian Brain

In the 1980s and 1990s, free D-amino acids were observed in mammalian tissues having various important functions. Free D-Ser was observed predominantly in the mammalian brain.[4-6] Hashimoto et al. suggested that D-Ser might regulate a NMDA/glycine receptor. Free D-Ser exists in the cerebrum and may function as an endogenous synaptic modulator. The localization and function of free D-Ser in the brain were reviewed in detail by Hashimoto.[7] After this discovery, Wolosker et al.[8] purified the serine racemase which catalyzes direct racemization from L-Ser to D-Ser. Serine racemase is the first enzyme isolated from mammals. D-Ser may be generated from L-Ser by the serine racemase and may be metabolized by the D-amino acid oxidase in the mammalian brain.

Free D-Aspartic Acid (Asp) in Various Mammalian Tissues

Dunlop et al.[9] reported a high concentration of free D-aspartic acid (D-Asp) in the neonatal cerebral hemisphere and adult pituitary of rats. Subsequently, several groups have also reported a high concentration of free D-Asp in the cortex and retina of chickens, rats, and humans during early development.[7,9,10] Surprisingly, the emergence of free D-Asp occurred in the brain and retina during an early developmental stage, but the content rapidly decreased thereafter until it was present only in trace amounts in adult tissue. For example, in the prefrontal cortex of the human brain, as much as 60% of the total Asp was in the D-form at week 14 of gestation, but rapidly decreased to trace levels by the time of birth.[7]

Most recently, the transient appearance of D-Asp was observed in endocrine organs, such as the testis,[5,11] adrenal,[5] and pineal glands.[12] In the pineal glands of an adult rat, both D-Asp and D-Ser were observed and the levels of D-Asp were as high as 30–40% of the total Asp content. The level of D-Asp was relatively low at age 2 weeks, increased significantly at age 4 to 10 weeks, and then gradually decreased up to age 36 weeks.[12] D-Asp is taken up into pinealocytes, the predominant melatonin-producing cells in the pineal gland, and may inhibit secretion of melatonin.[13] In adult rat testes, approximately 30% of total Asp is in the D-form.[5,11] The D-Asp content was relatively low in the testes of rats at age 3 weeks, but increased between the age of 6 and 10 weeks and then remained constant until age 40 weeks.[5] The D-Asp is localized in a region rich in elongate spermatids, the most mature form of germ cells.[5] Recent reports demonstrated that D-Asp is taken up into Leydig cells and may act on human chorionic gonadotropin (hCG)-induced testosterone synthesis, thereby increasing testosterone production.[14] Because of these observations, many researchers suggest that D-Asp may play a role as a novel messenger in the maturation and differentiation of these tissues. D-amino acid biosystems certainly exist in our living systems. Details of free D-Asp in the mammalian body are reviewed by Homma.[15]

Free D-Amino Acid in Various Marine Invertebrates

Free D-alanine was found in the tissues of several crustaceans and bivalve mollusks. Under high salinity stress, D- and L-alanine is accumulated in living organisms, therefore, the function of D-alanine is considered to be that of osmolytes.[16,17] Abe reviewed the advanced progress of the studies regarding the interesting functions of D-amino acids in higher marine organisms.[18]

D-Amino Acids in Peptides

D-Amino Acid Containing Peptides from Frogs

As described in the previous section, it was thought that D-amino acids containing peptides were limited to microorganisms. However, in recent years, small peptides containing one particular D-amino acid have been found in both vertebrates and invertebrates. Dermorphin is the first example of a D-amino acid-containing peptide in vertebrates. It was isolated from the skin secretions of a frog and is an opioid peptide with the sequence Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2.[19] Its biological activity is about 1000 times greater than that of morphine.[20] This activity is lost by substituting L-Ala for D-Ala, indicating that D-Ala is essential for its activity. The cloning of the precursor of dermorphin demonstrated that the structure of pro-dermorphin is consistent with a classical pro-hormone: it contains a signal peptide at its amino terminus, which is followed by repetitive segments of about 35 amino acids. This precursor encoded four copies of dermorphin plus one copy of another heptapeptide, Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2. The heptapeptide was named dermenkephalin because of its dermal origin and its enkephalin-like activity.[21] Subsequently, deltorphin I[22], Tyr-D-

Ala-Phe-Asp-Val-Val-Gly-NH_2 and deltorphin II, Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2 were also isolated from the same source and were found to exhibit an affinity and a selectivity for opioid receptors. The D-Ala present in dermorphin and deltorphin and the D-Met present in dermenkephalin were encoded by the usual L-Ala (GCG) and L-Met (ATG) codons.[21,23] In addition, these peptides have only been detected in the skin secretions of *Phyllomedusinae* from South and Central America but not in frogs from other regions.

D-Amino Acid Containing Peptides from Snails

Two different D-amino acid-containing peptides were isolated from snails. The first peptide, achatin I, Gly-D-Phe-Ala-Asp, was isolated from the ganglia and atrium of the African giant snail *Achatina fulica*.[24] Achatin-I enhances cardiac activity in two ways: centrally, this peptide increases impulse frequency and produces spike broadening of a heart excitatory neuron identified as PON; peripherally, it enhances amplitude and frequency of the heart beat. Achatin-I showed excitatory actions not only on the heart but on several other muscles.[25] The corresponding peptide with an L-Phe, termed achatin II, was also shown to exist in these tissues but it did not have any biological activity. The second peptide, fulicin, Phe-D-Asn-Glu-Phe-Val-NH_2, was isolated from the ganglia of the same snail and found to be a potent stimulator of the contraction of the penis retractor muscle.[26] As in the case of achatin I and the dermorphin family, the L-Asn-2-containing isomer of fulicin has no biological activity. The question of how D-amino acid residues are introduced specifically at position 2 in these peptides in both vertebrates and invertebrates is very interesting. However, the mechanism of epimerization is not yet known. The small peptides that contain D-amino acids are discussed in an excellent recent review.[27]

D-Amino Acid-Containing Peptides from Spiders

In addition to the small D-amino acid-containing peptides described in the previous sections, a longer peptide containing a D-amino acid was discovered by Kuwada et al.[28] This peptide was obtained from the toxin of a spider and was termed ω-agatoxin. It consists of 48 amino acid residues; the Ser at 46 was found to be a D-isomer. Its function is as a calcium channel blocker. Subsequently, isomerase activity from L-Ser to D-Ser was observed in the toxin of a spider.[29]

D-Amino Acids in Protein

The Presence of D-Amino Acids in Various Aged Human Tissues

Although proteins consist exclusively of L-amino acids, D-aspartic acid (D-Asp) has been detected in various tissues such as tooth[30–33], bone[34–36], aorta[37], brain[38–40], erythrocyte[41], eye lens[42–50], skin[51], ligament[52], and lung[53] from elderly individuals. D-serine (D-Ser) was found in the beta-amyloid protein of Alzheimer's disease (Table 1).[40,54] The presence of D-Asp in aged tissues of living organisms has been explained as a result of the racemization of aspartyl residues in the protein over time inasmuch as the proteins in such tissues are metabolically inert. The earlier studies almost exclusively simply documented the presence of D-Asp in whole tissues.

Recently, specific sites were identified in the human lens alpha A-crystallin[47], human lens alpha B-crystallin[48], and a beta-amyloid protein in the brain[40]. We have also studied the mechanism of the formation of D-Asp in a specific lens protein.[49,55,56] Table 1 lists the presence of D-amino acids in proteins from various tissues.

Table 1. The presence of D-amino acid in protein from various tissues

Tissue	Protein	Amino acid	Related disease	Specific sites	Reference
Tooth	phosphophoryn	D-Asp	?	?	33
Bone	osteocalcin	D-Asp	?	?	34
Bone	typeI collagen C-terminal tellopeptide	D-Asp	?	Asp 1211	36
Brain	myelin	D-Asp	?	?	38,39
Brain	β-amyloid	D-Asp	Alzheimer	Asp1, Asp7, Asp23	40
Brain	β-amyloid	D-Ser	Alzheimer	Ser 8, 26	54
Erythrocyte		D-Asp			41
Lens	αA-crystallin	D-Asp	Cataract	Asp58, Asp151	47
Lens	αB-crystallin	D-Asp	Cataract	Asp36, Asp62	48
Skin	elastin	D-Asp		?	51
Ligament	elastin	D-Asp		?	52
Lung	elastin	D-Asp		?	53
Aorta	elastin	D-Asp	Arteriosclelosis	?	37

Alpha A- and Alpha B-Crystallin in Lens Contains D-Asp Residues

Earlier studies showed that D-Asp accumulated in the proteins of the human lens with age.[42] However, because D-Asp was detected in homogenates of lenses, it could not be determined whether all of the aspartic acid in the lens protein was racemized uniformly, or whether particular aspartic acid residues with a greater tendency to racemization exist in specific lens proteins. We predicted that D-Asp residues might be present at some specific sites in some particular lens proteins. At first, human lenses were homogenized and the sample was subjected to DEAE-chromatography and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Subsequently, all proteins of the bands observed on SDS-PAGE were obtained by electronic elution and were hydrolyzed and analyzed for the D/L ratio of Asp. The protein with the highest D/L ratio of Asp has a molecular weight of approximately 20 kDa. Since the N-terminal of this protein was blocked, the protein was treated with trypsin; the resulting fragment was separated by RP-HPLC and analyzed. The D-Asp-containing protein was identified as alpha-crystallin.[43,44] Alpha-crystallin is a major structural protein of the lens, which is composed of alpha-, beta-, and gamma-crystallin. Racemization of Asp was observed in alpha-crystallin but not in beta- and gamma-crystallin. Alpha-crystallin has a molecular mass of 600–1000 kDa and it is comprised of two types of subunits, alpha A and alpha B, each with a molecular mass of approximately 20 kDa. We purified alpha A- and alpha B-crystallin from aged human lenses (age >80 years) and analyzed the racemization of the Asp residues in each protein. In aged human lenses, the D/L ratios of Asp residues in alpha A- and alpha B-crystallin were 0.19 and 0.11, respectively.[46] We next determined the D/L ratio of individual Asp and asparagine (Asn) residues in alpha A- and alpha B-crystallin.

Localization of D-Asp Residues in Alpha A- and Alpha B-Crystallin

Human alpha A- and alpha B-crystallin is composed of 173 and 175 amino acid residues, respectively (Figures 2a and 2b). Alpha A-crystallin has 15 Asp and 2 Asn residues while alpha B-crystallin contains 11 Asp and 3 Asn residues. As discussed in the previous section, the D/L ratios of total Asp in alpha A- and alpha B-crystallin of aged human lenses were estimated to be 0.19 and 0.11, respectively. Therefore, these values are considered to be the average value of 17 Asp (15 plus 2) residues of alpha A-crystallin and 14 Asp (11 plus 3) residues in alpha B-crystallin. If the specific Asp sites that are susceptible to racemization exist in the proteins, the D/L ratio of Asp should be higher than 0.19 or 0.11. Therefore, the purified alpha A- and alpha B-crystallins were digested with trypsin and the resulting peptides were separated by RP-HPLC in order to determine the D/L ratio of individual Asp and Asn residues in these proteins. This procedure was expected to yield 20 tryptic peptides, as shown in Figures 2a and 2b. Figure 3 shows a typical separation of the tryptic peptides of aged human alpha A-crystallin. Identification of peaks was based on amino acid sequence analysis and mass analysis. The peptides containing Asp/Asn residues are circled. These peptides were hydrolyzed with gas-phase 6N HCl for 7 h at 108°C and the samples were derivatized with *o*-phthalaldehyde (OPA) and *n*-tert-butyloxycarbonyl-L-cysteine to form diastereomers. The D/L ratio of amino acids was determined by RP-HPLC with a C18 column using fluorescence detection (344 nm as excitation and 433 nm as emission wavelength). Strikingly, in aged human alpha A-crystallin, two specific sites of Asp-58 and Asp-151 were found to be highly inverted to the L-enantiomer to D-enantiomers (D/L ratio: 3.1 for Asp-58, 5.7 for Asp-151). A D/L ratio higher than 1.0 is remarkable since it is not defined as racemization, but as the inversion of the configuration of L-Asp to D-Asp. This is the first observation of the inversion of amino acid residues in protein.[47] The reason for the forming of predominantly D-Asp will be described later. Conversely, two specific sites, Asp-36 (D/L ratio of Asp: 0.92) and Asp-62 (D/L ratio of Asp: 0.57) were found to be highly racemized. These results are summarized in Table 2. In both alpha A- and alpha B-crystallin, the stereoconfiguration conversion of Asp, i.e., L-Asp to D-Asp, accompanied isomerization, which forms beta-Asp residues. The peptides containing the beta- and alpha-Asp residues were clearly separated by RP-HPLC, that is, the beta-Asp-containing peptide eluted faster than the alpha-Asp-containing peptide did. The beta-Asp-containing peptide was identified by a protein sequencer because the abnormal peptide is resistant to Edman degradation.

Mechanism of D-Asp and Beta-Asp Formation in Protein

In alpha A- and alpha B-crystallin, the D-Asp and beta-Asp formation occurred simultaneously. This result indicates that D-Asp formation in protein occurs via a succinimide intermediate. As shown in Figure 4, the simultaneous formation of beta- and D-Asp residues in the protein could be explained as follows: (1) When the carbonyl group of the side chain of the L-alpha-aspartyl residue is attacked by the nitrogen of the amino acid residue following the Asp residue, L-succinimide is formed by intramolecular cyclization; (2) L-succinimide may be converted to D-succinimide through an intermediate [I] that has the prochiral alpha-carbon in the plane of the ring; (3) Protonation of the intermediate [I] would occur with equal probability from the upper or lower side of the plane in an ordinary peptide or protein (racemization); (4) D- and L-succinimide are hydrolyzed at either side of their two carbonyl groups, yielding both beta- and alpha-Asp residues, respec-

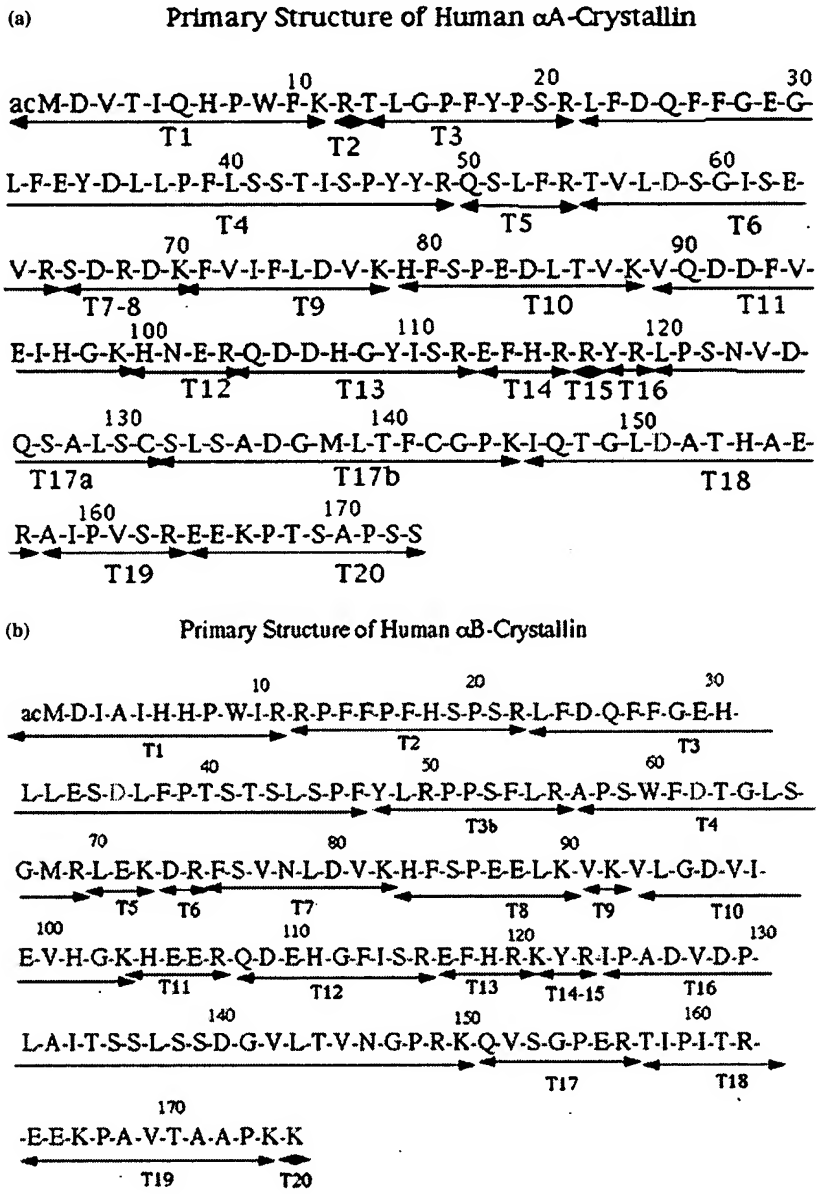


Fig. 2. (a) Primary structure of human alpha A-crystallin. (b) Primary structure of human alpha B-crystallin.

tively. The rate of succinimide formation is expected to depend on the neighboring residue of the Asp residue. When the neighboring amino acid of the Asp residue has a small side chain, such as glycine, alanine, or serine, the formation of succinimide occurs easily because there is no steric hindrance.[57-59] We synthesized three peptides corresponding to fragments of alpha A-crystallin (T18, T6, and T10 in Figure 2a) and carried out kinetic studies of the racemization of Asp in these peptides. The results indicated that the Asp residue in peptide T18 (Asp-151) was the most susceptible to racemization, while the Asp residue in peptide T10 (Asp-84) was the least susceptible. The racemization rate of Asp decreases according to the level of steric hindrance of the carboxyl side chain of the Asp residue.[55] This order of susceptibility is consistent with that of native human alpha A-crystallin. However, a very important difference concerning D-Asp formation in the model peptide

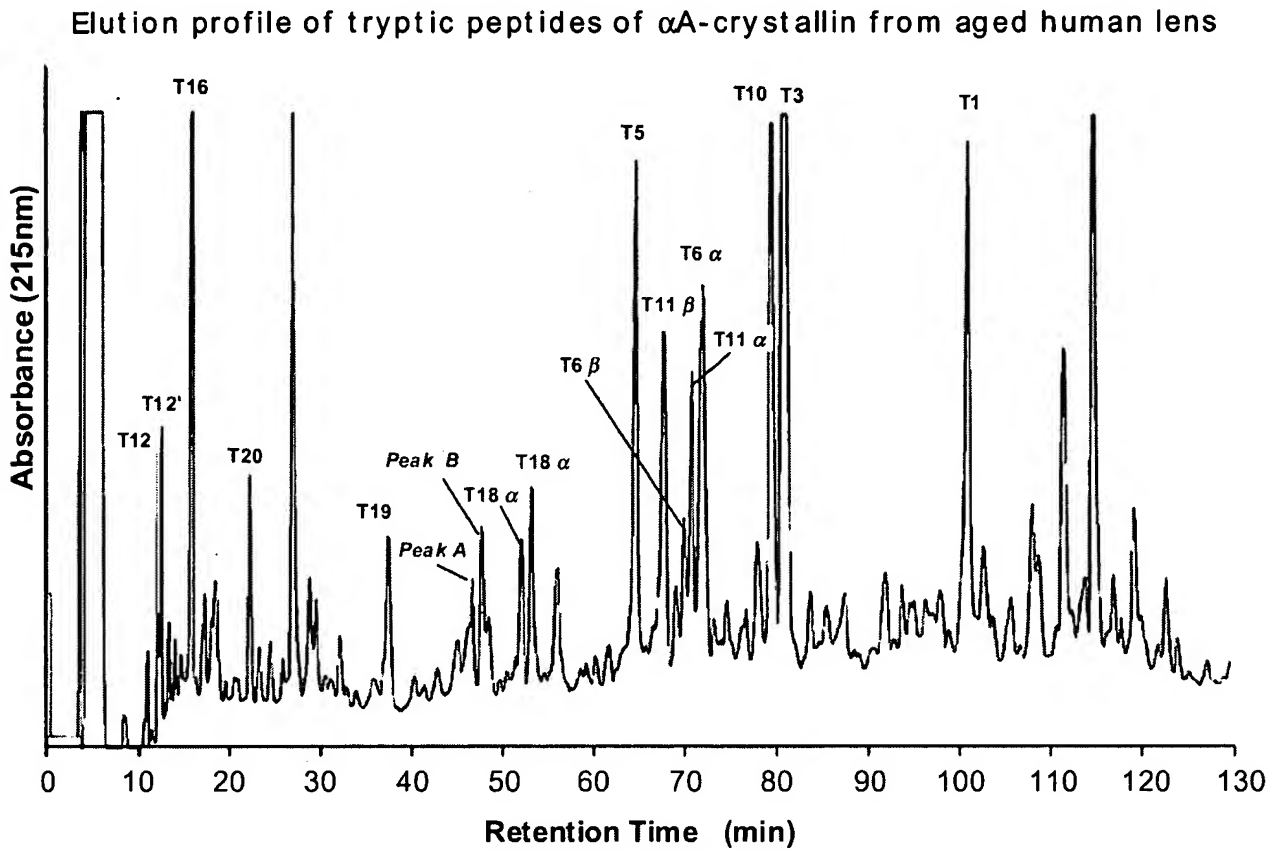


Fig. 3. Elution profiles of tryptic (T) peptides of alpha A-crystallin from the lenses of elderly donors (80-years-old). The peptides were separated by RP-HPLC using a C18 column (TSK gel-ODS-80 TM, 4.6 × 250 mm, Tosoh, Tokyo) with a linear gradient of 0–40% acetonitrile, 0.1% trifluoroacetic acid, at a flow rate of 1 ml/min over 120 min. Peptides were detected by measuring their absorbance at 215 nm. The assignment of each peak was based on amino acid composition, sequence analysis, and mass analysis.

and in the native protein is that inversion (D/L > 1.0) of the L-Asp residues occurred in the native protein but not in the short model peptide. In the native protein, we found that the D/L ratios of the Asp-151 and Asp-58 residues in the 80-year-old human alpha A-crystallin were much higher than 1.0 (D/L ratio: Asp-151 = 5.7, Asp-58 = 3.1, respectively) (Table 2). Since racemization is defined as a reversible first-order reaction, when the D/L ratio reaches 1.0, the racemization is at equilibrium. Thus, the D/L ratios that were greater than 1.0 would not be defined as racemization, but as the inversion of L-Asp to its D-isomer. In the short model peptides, the racemization (0 < D/L < 1.0) of the Asp residue proceeded normally but the stereoinversion of the Asp residue did not occur. This result suggests that the area surrounding the Asp-151 and Asp-58 residues might form a chiral environment that allows the inversion of L-Asp residues to D-Asp residues in alpha A-crystallin.

Why Does Stereoinversion of Asp Occur in Protein?

The stereoinversion (D/L of Asp > 1.0) is caused from the chiral field that is itself composed of protein.

As described in the previous section, the D/L ratios of the Asp residues in the model peptide did not exceed 1.0. In other words, D-Asp formation in unstructured peptides proceeded according to the theory of racemization. This suggests that a chiral reaction field exists in the native higher order structure of human alpha A-crystallin that induces the inversion of L-Asp to D-Asp residues. If such a chiral reaction field does indeed exist in the native structure of the protein, we speculated that the rate of inversion of Asp-151 should decrease in unfolded alpha A-crystallin due to the disappearance of the chiral environment. Conversely, we reasoned that upon the unfolding of alpha A-crystallin from an aged human lens,

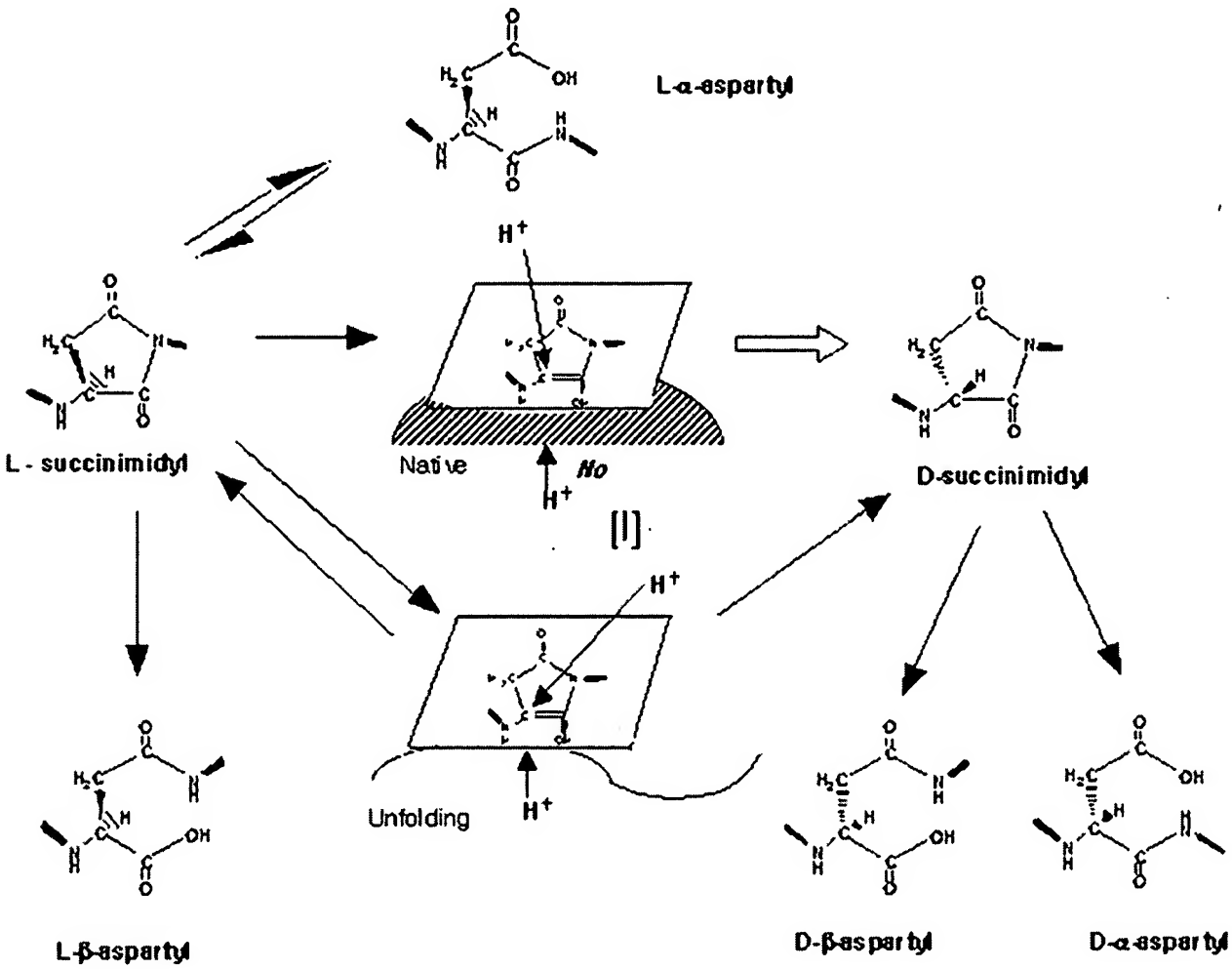


Fig. 4. Reaction pathways for spontaneous inversion and isomerization of aspartyl residues in protein. The possible structural environment surrounding the intermediate [I] that induces the inversion to the D-isomer, is shown. A local structure, which hinders the protonation from the lower side, may be present beneath the ring plane of the intermediate [I] (shaded), resulting in the protonation of the intermediate I from the upper side of the plane, and causing inversion of the configuration to the D-form.

Table 2. Stereoinversion and isomerization of αA-, αB-crystallin from aged human lens.

Crystallin	Asp	D/L of Asp	Linkage
αA	Asp-58	3.10	beta
αA	Asp-151	5.70	beta
αB	Asp-36	0.92	beta
αB	Asp-62	0.57	beta

which is enriched for the D-form at Asp-151, the D-form might be converted to the L-form, implying that a chiral field exists in the folded protein that initially induces the specific stereoinversion. Therefore, we unfolded the alpha A-crystallin obtained from the aged human lens to a random coil structure by exposure to 6M urea and measured the D/L ratio of the Asp-151 residue.[56] In the native alpha A-crystallin, the D/L ratio of the beta-linked Asp-151 residue was 5.7, while the D/L ratio of the beta-linked Asp-151 residues in unfolded alpha A-crystallin approached approximately 1.0. These results strongly indicated that a chiral reaction field exists in the native higher

order structure of human alpha-A-crystallin that induces the inversion of L-Asp to D-Asp residues. As shown in Figure 4, the protonation of the intermediate [I] does not occur with equal probability from the upper or lower side of the plane. Namely, a sterically hindering structure composed of the native higher order structure of alpha A-crystallin might be present on the lower side of the intermediate [I] (Figure 4 shaded parts), resulting in the protonation of the intermediate [I] from the upper side of the plane. This, in turn, causes the configuration to be inverted to the D-form (Figure 4). Conversely, when the native higher order structure is destroyed by urea, protonation to the intermediate [I] could occur with an equal probability from either side of the plane, showing the D/L of Asp = 1.0. This result indicates that the structural field that surrounds the Asp-151 residue induces the formation of D-beta-Asp, and that this field is composed of the higher-order conformation of alpha A-crystallin. Where is the chiral field that predominantly induces D-Asp in the part of alpha A-crystallin?

Recently, we identified truncated peptides formed by a post-translational cleavage event between His-154 and Ala-155 residues in aged alpha A-crystallin protein.[49] The D/L ratio of Asp-151 in the truncated alpha A-crystallin 1-154 was 0.3. Unlike the native full-length (1-173) alpha A-crystallin, the stereoinversion of Asp-151 was not observed in the cleaved 1-154 polypeptide from alpha A-crystallin. Considered together with the above results, the chiral reaction field of native human alpha A-crystallin might consist of the region from Ala-155 to the C-terminus residue along with other residues close to the C-terminus.

The stereoinversion and isomerization of the Asp residues occur simultaneously. Therefore, four isomers, which are normal L-alpha-Asp, biologically uncommon L-beta-Asp, D-alpha-Asp, and D-beta-Asp, are formed in alpha A-crystallins. Recently, we measured the ratio of the four isomers of Asp-151 in alpha A-crystallins obtained from human lenses of the newborn, 30-, 60-, and 80-year-old ranges.[60] As shown in Figure 5, the isomers increased with age, and the total amount of the three isomers was greater than that of normal L-alpha-Asp in the alpha A-crystallin of the human lenses of the 80-year-old range. The drastic changes started at birth, with about 45% of normal L-alpha-Asp-151 lost by the 30-year-old range.

D-Amino Acid Containing Protein in Other Tissues

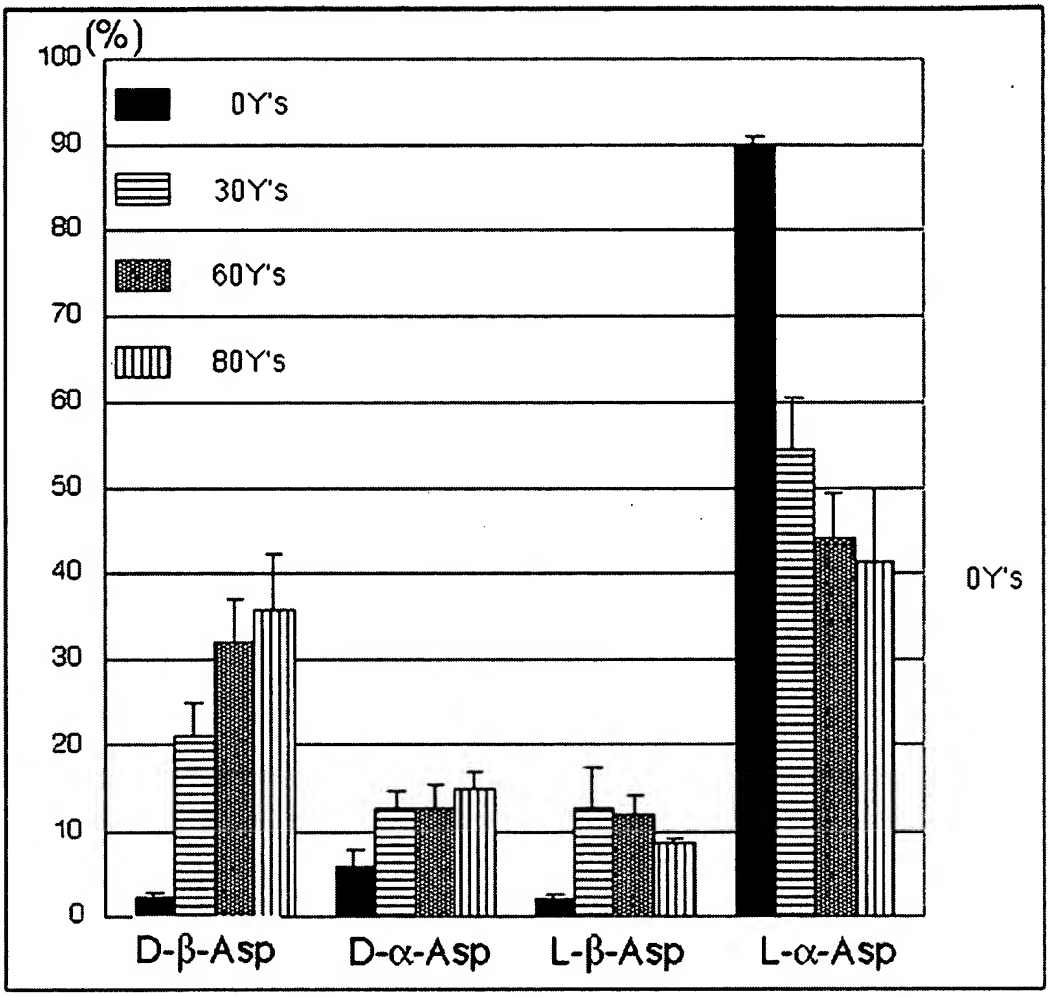
As discussed in the sections above, the D-Asp formation occurs much more easily in proteins than was thought. Recently, we found D-beta-Asp-containing protein in the elastic fibers of skin from elderly donors.[51] The formation of D-beta-Asp in protein related not only to aging but also to UV irradiation. Figure 6 clearly indicates that D-beta-Asp-containing protein was observed only in the facial skin of elderly donors (Figures 6b and 6c), but was not detected in the facial skin of young donors (Figure 6a), nor in sun-protected skin (buttocks) of elderly donors (Figure 6d). These results suggest that D-beta-Asp-containing protein is accelerated by exposure to sunlight and that the exceptional protein accumulates in aged skin during UV-related aging. As shown in Table 1, the number of reports concerning the presence of D-Asp residues in various proteins has rapidly increased. The localization of D-Asp in these proteins will undoubtedly be clarified by further research.

Prospects

Recent studies indicate that D-amino acids are present in peptides and proteins quite extensively, as well as in the form of free D-amino acids in various living tissues. What is the origin of these D-amino acids in the living body? What is the physiological significance of D-amino acids in the living body? It is said that 3.5 billion years of evolution led to the source of human and animal life. Therefore, the emergence of free D-aspartic acid in early development and the drastic decreases of free D-amino acids after birth, may be correlated with the earth's chemical evolution before the emergence of life. The fluctuation of the amount of free D-amino acids in the living body suggests that D-amino acids might be one of the factors controlling the generation and differentiation of cells or tissues. Although these problems have barely been considered, it is a very interesting theme. Further, more detailed studies are required to elucidate the physiological meaning of free D-amino acid.

In another light, D-amino acids in proteins can be interpreted as molecular markers of aging. Most researchers have held that L-amino acids in proteins could never change to D-isomers under the physical conditions of the living body because protein may not be easily modifiable chemically, because evolutionary selection has operated to ensure the very stable properties of such molecules. This general idea had no real basis in scientific fact but became established because D-amino acids could not be found. However, recent improvements in analytical techniques now enable accurate analysis of amino acid enantiomers at the picomole level. Therefore, we are able to identify a very small quantity of D-aspartic acid at specific sites in lens proteins consisting almost entirely of L-amino acids—a task similar to looking for a needle in a haystack.

Aspartic acid residues are not racemized uniformly but racemization proceeds at specific protein sites such as alpha-crystallin and beta-amyloid protein, which were examined. We were also able to propose a mechanism for D- and beta-aspartic acid formation in alpha A-crystallin. The formation of



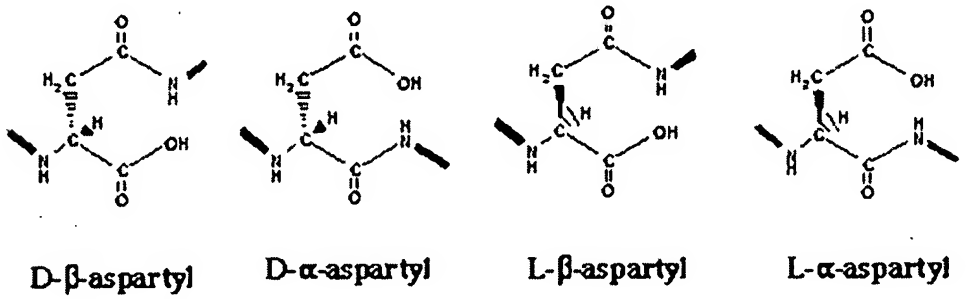


Fig. 5. Relative amounts of four optical isomers of Asp-151 residues, which are normal L-alpha-Asp, biologically exceptional L-beta-Asp, D-alpha-Asp, and D-beta-Asp in alpha A-crystallins from 0-, 30-, 60-, 80-year-old ranges of donor lenses.

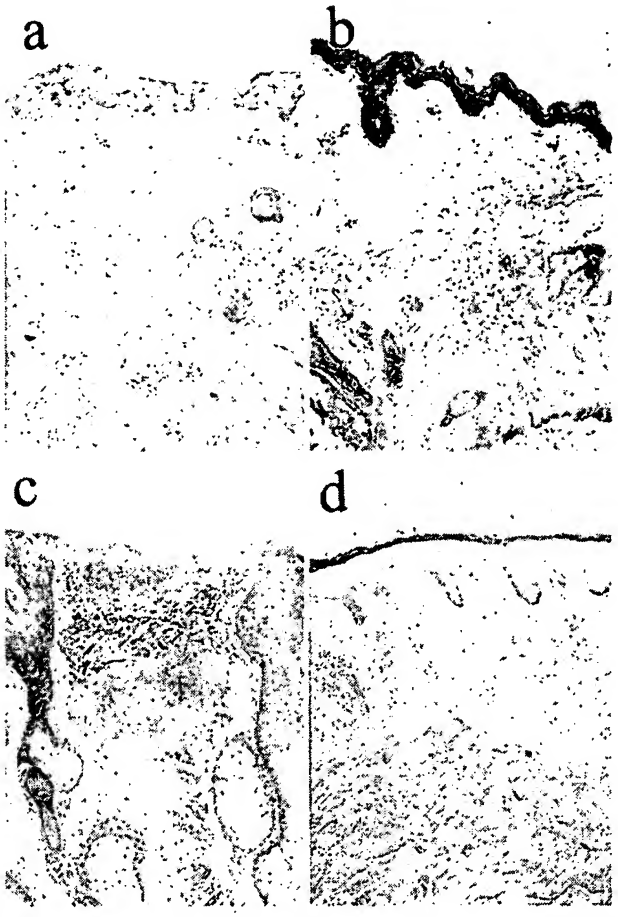


Fig. 6. Immunohistochemistry of the antibody for D-beta-Asp-containing peptide in the skin. Skin specimens obtained from the sun-exposed area (face) of (a) 9-year-old, (b) 59-year-old, and (c) 86-year-old, or (d) the sun-protected area (buttock) of 85-year-old.

D-aspartic acids in protein depends on the primary structure and the higher-order structure surrounding the Asp residues. Notably, the D/L ratios of the Asp-151 and Asp-58 residues in alpha A-crystallin were higher than 1.0. Since racemization is defined as a reversible first-order reaction, when the D/L ratio reaches 1.0, racemization is in equilibrium. Therefore, this reaction must be considered an unprecedented stereoinversion reaction, and not ordinary racemization. We propose that a chiral reaction field exists in the native higher-order structure of human alpha A-crystallin that induces the inversion of L-Asp to D-Asp residues at Asp-151.

The formation of D-amino acid with age partially proceeds in proteins that contain only one-handed structures consisting of L-amino acids in an evolutionary process opposite to the evolution of life. The appearance of D-amino acids in aging and the presence of free D-amino acids before birth may embody the origin and evolution of life in individual living bodies.

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